

SEQUENCE LISTING

<110> LIN, LEU-FEN H

COLLINS, FRANKLIN D

DOHERTY, DANIEL H

LILE, JACK

BEKTESH, SUSAN

<120> Glial Cell Line-Derived Neurotrophic Factor

<130> S-225E Rev

<140> 08/182,183

<141> 1994-05-23

<150> 07/764,685

<151> 1991-09-20

<150> 07/774,109

<151> 1991-10-08

<150> 07/788,423

<151> 1991-11-06

<150> 07/855,413

<151> 1992-03-19

<150> PCT/US92/07888

<151> 1992-09-17

<160> 28

<170> PatentIn version 3.1

<210> 1

<211> 25

<212> PRT

<213> Rattus rattus

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<221> MISC_FEATURE

<222> (16)..(16)

<223> Xaa in position 16 may be any one of the 20 naturally occurring amino acids.

<220>

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<223> N-terminal fragment

<400> 1

Ser	Pro	Asp	Lys	Gln	Ala	Ala	Ala	Leu	Pro	Arg	Arg	Glu	Arg	Asn	Xaa
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Gln	Ala	Ala	Ala	Ala	Ser	Pro	Asp	Asn							
			20					25							

<210> 2

<211> 13

<212> PRT

<213> Rattus rattus

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<222> (2)..(2)

<223> Xaa in position 2 is either Lys or Gln

<220>

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<223> Internal GDNF peptide fragment

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Asp	Xaa	Ile	Leu	Lys	Asn	Leu	Gly	Arg	Val	Arg	Arg	Leu
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<210> 3

<211> 900

<212> DNA

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<220>

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<222> (25)..(705)

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<221> mat_peptide

<222> (304)..()

<223>

<400> 3

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 Val Tyr Gly Asp Arg Ile Arg Gly Ala
 -90 -85

gcc gcc gga cgg gac tct aag atg aag tta tgg gat gtc gtg gct gtc 99
 Ala Ala Gly Arg Asp Ser Lys Met Lys Leu Trp Asp Val Val Ala Val
 -80 -75 -70

tgc ctg gtg ttg ctg cac acc gcg tct gcc ttc ccg ctg ccc gcc ggt 147
 Cys Leu Val Leu Leu His Thr Ala Ser Ala Phe Pro Leu Pro Ala Gly
 -65 -60 -55

aag agg ctt ctc gaa gcg ccc gcc gaa gac cac tcc ctc ggc cac cgc 195
 Lys Arg Leu Leu Glu Ala Pro Ala Glu Asp His Ser Leu Gly His Arg
 -50 -45 -40

cgc gtg ccc ttc gcg ctg acc agt gac tcc aat atg ccc gaa gat tat 243
 Arg Val Pro Phe Ala Leu Thr Ser Asp Ser Asn Met Pro Glu Asp Tyr
 -35 -30 -25

cct gac cag ttt gat gac gtc atg gat ttt att caa gcc acc atc aaa 291
 Pro Asp Gln Phe Asp Asp Val Met Asp Phe Ile Gln Ala Thr Ile Lys
 -20 -15 -10 -5

aga ctg aaa agg tca cca gat aaa caa gcg gcg gca ctt cct cga aga 339
 Arg Leu Lys Arg Ser Pro Asp Lys Gln Ala Ala Ala Leu Pro Arg Arg
 -1 1 5

gag agg aac cgg caa gct gca gct gcc agc cca gag aat tcc aga ggg 387
 Glu Arg Asn Arg Gln Ala Ala Ala Ser Pro Glu Asn Ser Arg Gly
 15 20 25

aaa ggt cgc aga ggc cag agg ggc aaa aat cgg ggg tgc gtc tta act 435
 Lys Gly Arg Arg Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr
 30 35 40

gca ata cac tta aat gtc act gac ttg ggt ttg ggc tac gaa acc aag 483
 Ala Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys
 45 50 55 60

gag gaa ctg atc ttt cga tat tgt agc ggt tcc tgt gaa gcg gcc gag 531
 Glu Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys Glu Ala Ala Glu
 65 70 75

aca atg tac gac aaa ata cta aaa aat ctg tct cga agt aga agg cta 579
 Thr Met Tyr Asp Lys Ile Leu Lys Asn Leu Ser Arg Ser Arg Arg Leu
 80 85 90

aca agt gac aag gta ggc cag gca tgt tgc agg ccg gtc gcc ttc gac 627
 Thr Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro Val Ala Phe Asp
 95 100 105

gac gac ctg tcg ttt tta gac gac agc ctg gtt tac cat atc cta aga 675
 Asp Asp Leu Ser Phe Leu Asp Asp Ser Leu Val Tyr His Ile Leu Arg
 110 115 120

aag cat tcc gct aaa cgg tgt gga tgt atc tgaccctggc tccagagact 725
 Lys His Ser Ala Lys Arg Cys Gly Cys Ile
 125 130

gctgtgtatt gcattcctgc tacactgcga agaaagggac caaggttccc aggaaatatt 785

tgcccagaaa ggaagataag gaccaagaag gcagaggcag aggcggaaga agaagaagaa 845

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<210> 4

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<212> PRT

<213> Rattus rattus

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Met Lys Leu Trp Asp Val Val Ala Val Cys Leu Val Leu Leu His Thr
 -75 -70 -65

Ala Ser Ala Phe Pro Leu Pro Ala Gly Lys Arg Leu Leu Glu Ala Pro
 -60 -55 -50

Ala Glu Asp His Ser Leu Gly His Arg Arg Val Pro Phe Ala Leu Thr
 -45 -40 -35 -30

Ser Asp Ser Asn Met Pro Glu Asp Tyr Pro Asp Gln Phe Asp Asp Val
 -25 -20 -15

Met Asp Phe Ile Gln Ala Thr Ile Lys Arg Leu Lys Arg Ser Pro Asp
 -10 -5 -1 1

Lys Gln Ala Ala Ala Leu Pro Arg Arg Glu Arg Asn Arg Gln Ala Ala
 5 10 15

Ala Ala Ser Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg Gly Gln Arg
 20 25 30 35

Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu Asn Val Thr
 40 45 50

Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile Phe Arg Tyr
55 60 65

Cys Ser Gly Ser Cys Glu Ala Ala Glu Thr Met Tyr Asp Lys Ile Leu
70 75 80

Lys Asn Leu Ser Arg Ser Arg Arg Leu Thr Ser Asp Lys Val Gly Gln
85 90 95

Ala-Cys Cys Arg Pro Val Ala-Phe-Asp-Asp-Asp Leu Ser Phe Leu Asp
100 105 110 115

Asp Ser Leu Val Tyr His Ile Leu Arg Lys His Ser Ala Lys Arg Cys
120 125 130

Gly Cys Ile

<210> 5

<211> 562

<212> DNA

<213> Homo sapiens

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<222> (105)..()

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-25 -20

ttc gat gat gtc atg gat ttt att caa gcc acc att aaa aga ctg aaa 101
Phe Asp Asp Val Met Asp Phe Ile Gln Ala Thr Ile Lys Arg Leu Lys
-15 -10 -5

agg tca cca gat aaa caa atg gca gtg ctt cct aga aga gag cgg aat 149
Arg Ser Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu Arg Asn
-1 1 5 10 15

cgg cag gct gca gct gcc aac cca gag aat tcc aga gga aaa ggt cgg 197
Arg Gln Ala Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg
20 25 30

aga ggc cag agg ggc aaa aac cgg ggt tgt gtc tta act gca ata cat 245
 Arg Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His
 35 40 45
 tta aat gtc act gac ttg ggt ctg ggc tat gaa acc aag gag gaa ctg 293
 Leu Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu
 50 55 60
 att ttt agg tac tgc agc ggc tct tgc gat gca gct gag aca acg tac 341
 Ile Phe Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr
 65 70 75
 gac aaa ata ttg aaa aac tta tcc aga aat aga agg ctg gtg act gac 389
 Asp Lys Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val Thr Asp
 80 85 90 95
 aaa gta ggg cag gca tgt tgc aga ccc atc gcc ttt gat gat gac ctg 437
 Lys Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp Asp Leu
 100 105 110
 tcg ttt tta gat gat aac ctg gtt tac cat att cta aga aag cat tcc 485
 Ser Phe Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys His Ser
 115 120 125
 gct aaa agg tgt gga tgt atc tgactccggc tccagagact gctgtgtatt 536
 Ala Lys Arg Cys Gly Cys Ile
 130
 gcattcctgc tacagtgcaa agaaag 562

<210> 6

<211> 161

<212> PRT

<213> Homo sapiens

<400> 6

Ser Asn Met Pro Glu Asp Tyr Pro Asp Gln Phe Asp Asp Val Met Asp
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 Phe Ile Gln Ala Thr Ile Lys Arg Leu Lys Arg Ser Pro Asp Lys Gln
 -10 -5 -1 1 5
 Met Ala Val Leu Pro Arg Arg Glu Arg Asn Arg Gln Ala Ala Ala Ala
 10 15 20
 Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg Gly Gln Arg Gly Lys
 25 30 35
 Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu Asn Val Thr Asp Leu
 40 45 50
 Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile Phe Arg Tyr Cys Ser
 55 60 65
 Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr Asp Lys Ile Leu Lys Asn
 70 75 80 85
 Leu Ser Arg Asn Arg Arg Leu Val Thr Asp Lys Val Gly Gln Ala Cys

	90		95		100
Cys	Arg	Pro	Ile	Ala	Phe
	105		110		115
Leu	Val	Tyr	His	Ile	Leu
	120		125		130

Ile

<210> 7

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide probe

<220>

<221> misc_feature

<222> (3)..(3)

<223> N at position 3 is inosine

<220>

<221> misc_feature

<222> (15)..(15)

<223> N at position 15 is inosine

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<221> misc_feature

<222> (18)..(18)

<223> N at position 18 is inosine

<400> 7

ccngayaarc argcngcngc

20

<210> 8

<211> 223

<212> DNA

<213> Homo sapiens

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 ttctctcccc cacctcccgc ctgcccgcgc aggtgccgcc gccggacggg actttaagat 60
 gaagttatgg gatgtcgtgg ctgtctgcct ggtgctgctc cacaccgct cgccttccc 120
 gctgcccgcc ggtaagaggg ctcccagggc gcccgccgaa gaccgctccc tcggccgccc 180
 ccgcgcgccc ttcgcgctga gcagtgactg taagaaccgt tcc 223

<210> 9

<211> 12

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide linker

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 cccgaattcg gg 12

<210> 10

<211> 7

<212> PRT

<213> Rattus rattus

<400> 10

Pro Asp Lys Gln Ala Ala Ala
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<210> 11

<211> 33

<212> DNA

<213> Artificial sequence

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<223> Nucleic acid sequence from pBluescript SK-76.1 encoding rat GDNF
 N-terminus sequence

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 gagaggaacc ggcaagctgc wgmwgywmwgm ccw 33

<210> 12

<211> 11

<212> PRT

<213> Rattus rattus

<400> 12

Glu Arg Asn Arg Gln Ala Ala Ala Ala Ser Pro
1 5 10

<210> 13

<211> 20

<212> DNA

<213> Artificial sequence

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<223> Oligonucleotide PCR primer DHD-26 to amplify DNA encoding rat GDNF polypeptide

<220>

<221> misc_feature

<222> (9)..(9)

<223> N at position 9 is inosine

<220>

<221> misc_feature

<222> (12)..(12)

<223> N at position 12 is inosine

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<210> 14

<211> 7

<212> PRT

<213> Rattus rattus

<220>

<221> misc_feature

<223> Internal rat GDNF peptide

<400> 14

Asp Lys Ile Leu Lys Asn Leu
1 5

<210> 15

<211> 17

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide primer PD1 to amplify rat GDNF probe

<400> 15
gacgggactc taagatg

17

<210> 16

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer DHD23 to amplify rat GDNF probe

<220>

<221> misc_feature

<222> (3)..(3)

<223> N at position 3 is inosine

<220>

<221> misc_feature

<222> (6)..(6)

<223> N at position 3 is inosine

<220>

<221> misc_feature

<222> (18)..(18)

<223> N at position 3 is inosine

<400> 16
gcngcngcyt gyttrtcngg

20

<210> 17

<211> 17

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide primer LF2 to amplify rat GDNF probe

<400> 17
cgagacaatg tacgaca

17

<210> 18

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer PD2 to amplify rat GDNF probe

<400> 18
ctctggagcc aggggtca

17

<210> 19

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer PD1 to amplify rat GDNF probe

<400> 19
cccgaattcg acgggactct aagatg

26

<210> 20

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer LFA to amplify rat GDNF probe

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cggtggccag agggagtggt cttc

24

<210> 21

<211> 46

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide primer PD3 to amplify human cDNA

<400> 21

cgcggtacca ataaggagga aaaaaaatgt caccagataa acaaat

46

<210> 22

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer PD4 to amplify human cDNA

<400> 22

cgcggtaccc agtctctgga gccgga

26

<210> 23

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic adapter fragment for plasmid pCJ1

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gatctagaat tgtcatgttt gacagcttat cat

33

<210> 24

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Polylinker sequence for plasmid pCJX1-1 with EcoRI and PSTI overhangs

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aattcccggt taccagatct gagctcacta gtctgca

37

<210> 25

<211> 747

<212> DNA

<213> Homo sapiens

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-85 -80

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Phe Lys Met Lys Leu Trp Asp Val Val Ala Val Cys Leu Val Leu Leu
-75 -70 -65

cac acc gcg tcc gcc ttc ccg ctg ccc gcc ggt aag agg cct ccc gag 148
His Thr Ala Ser Ala Phe Pro Leu Pro Ala Gly Lys Arg Pro Pro Glu
-60 -55 -50

gcg ccc gcc gaa gac cgc tcc ctc gcc cgc cgc cgc gcg ccc ttc gcg 196
Ala Pro Ala Glu Asp Arg Ser Leu Gly Arg Arg Arg Ala Pro Phe Ala
-45 -40 -35

ctg agc agt gac tca aat atg cca gag gat tat cct gat cag ttc gat 244
Leu Ser Ser Asp Ser Asn Met Pro Glu Asp Tyr Pro Asp Gln Phe Asp
-30 -25 -20

gat gtc atg gat ttt att caa gcc acc att aaa aga ctg aaa agg tca 292
Asp Val Met Asp Phe Ile Gln Ala Thr Ile Lys Arg Leu Lys Arg Ser

-15	-10	-5	-1	1	
cca gat aaa caa atg gca gtg ctt cct aga aga gag cgg aat cgg cag					340
Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu Arg Asn Arg Gln					
5		10		15	
gct gca gct gcc aac cca gag aat tcc aga gga aaa ggt cgg aga ggc					388
Ala Ala Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg Gly					
20		25		30	
cag agg ggc aaa aac cgg ggt tgt gtc tta act gca ata cat tta aat					436
Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu Asn					
35		40		45	
gtc act gac ttg ggt ctg ggc tat gaa acc aag gag gaa ctg att ttt					484
Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile Phe					
50		55		60	65
agg tac tgc agc ggc tct tgc gat gca gct gag aca acg tac gac aaa					532
Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr Asp Lys					
70		75		80	
ata ttg aaa aac tta tcc aga aat aga agg ctg gtg act gac aaa gta					580
Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val Thr Asp Lys Val					
85		90		95	
ggg cag gca tgt tgc aga ccc atc gcc ttt gat gat gac ctg tcg ttt					628
Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp Asp Leu Ser Phe					
100		105		110	
tta gat gat aac ctg gtt tac cat att cta aga aag cat tcc gct aaa					676
Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys His Ser Ala Lys					
115		120		125	
agg tgt gga tgt atc tgactccggc tccagagact gctgtgtatt gcattcctgc					731
Arg Cys Gly Cys Ile					
130					
tacagtgcaa agaaag					747
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Gly Ala Ala Ala Gly Arg Asp Phe Lys Met Lys Leu Trp Asp Val Val					
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Ala Val Cys Leu Val Leu Leu His Thr Ala Ser Ala Phe Pro Leu Pro					
-70		-65		-60	-55
Ala Gly Lys Arg Pro Pro Glu Ala Pro Ala Glu Asp Arg Ser Leu Gly					
-50		-45		-40	
Arg Arg Arg Ala Pro Phe Ala Leu Ser Ser Asp Ser Asn Met Pro Glu					
-35		-30		-25	

Asp	Tyr	Pro	Asp	Gln	Phe	Asp	Asp	Val	Met	Asp	Phe	Ile	Gln	Ala	Thr
		-20					-15					-10			
Ile	Lys	Arg	Leu	Lys	Arg	Ser	Pro	Asp	Lys	Gln	Met	Ala	Val	Leu	Pro
	-5				-1	1				5					10
Arg	Arg	Glu	Arg	Asn	Arg	Gln	Ala	Ala	Ala	Ala	Asn	Pro	Glu	Asn	Ser
				15					20					25	
Arg	Gly	Lys	Gly	Arg	Arg	Gly	Gln	Arg	Gly	Lys	Asn	Arg	Gly	Cys	Val
			30					35					40		
Leu	Thr	Ala	Ile	His	Leu	Asn	Val	Thr	Asp	Leu	Gly	Leu	Gly	Tyr	Glu
		45					50					55			
Thr	Lys	Glu	Glu	Leu	Ile	Phe	Arg	Tyr	Cys	Ser	Gly	Ser	Cys	Asp	Ala
	60					65					70				
Ala	Glu	Thr	Thr	Tyr	Asp	Lys	Ile	Leu	Lys	Asn	Leu	Ser	Arg	Asn	Arg
75					80					85					90
Arg	Leu	Val	Thr	Asp	Lys	Val	Gly	Gln	Ala	Cys	Cys	Arg	Pro	Ile	Ala
				95					100					105	
Phe	Asp	Asp	Asp	Leu	Ser	Phe	Leu	Asp	Asp	Asn	Leu	Val	Tyr	His	Ile
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Leu	Arg	Lys	His	Ser	Ala	Lys	Arg	Cys	Gly	Cys	Ile				
		125					130								

<210> 27

$\langle 211 \rangle$ 211

<212> PRT

<213> Rattus rattus

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<221> MISC_FEATURE

<223> Rat pre-pro GDNF

<400> 27

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Ala	Ser	Ala	Phe	Pro	Leu	Pro	Ala	Gly	Lys	Arg	Leu	Leu	Glu	Ala	Pro
			20					25					30		
Ala	Glu	Asp	His	Ser	Leu	Gly	His	Arg	Arg	Val	Pro	Phe	Ala	Leu	Thr
			35				40					45			
Ser	Asp	Ser	Asn	Met	Pro	Glu	Asp	Tyr	Pro	Asp	Gln	Phe	Asp	Asp	Val
	50					55					60				
Met	Asp	Phe	Ile	Gln	Ala	Thr	Ile	Lys	Arg	Leu	Lys	Arg	Ser	Pro	Asp
65					70					75					80

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<210> 28
<211> 211
<212> PRT
<213> Homo sapiens
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<223> Human pre-pro GDNF
<400> 28
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Ala	Glu	Asp 35	Arg	Ser	Leu	Gly	Arg 40	Arg	Arg	Ala	Pro	Phe 45	Ala	Leu	Ser
Ser	Asp 50	Ser	Asn	Met	Pro	Glu 55	Asp	Tyr	Pro	Asp	Gln 60	Phe	Asp	Asp	Val
Met 65	Asp	Phe	Ile	Gln	Ala 70	Thr	Ile	Lys	Arg	Leu 75	Lys	Arg	Ser	Pro	Asp 80
Lys	Gln	Met	Ala	Val 85	Leu	Pro	Arg	Arg	Glu 90	Arg	Asn	Arg	Gln	Ala 95	Ala

Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg Gly Gln Arg
100 105 110

Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu Asn Val Thr
115 120 125

Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile Phe Arg Tyr
130 135 140

Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr Asp Lys Ile Leu
145 150 155 160

Lys Asn Leu Ser Arg Asn Arg Arg Leu Val Thr Asp Lys Val Gly Gln
165 170 175

Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp Asp Leu Ser Phe Leu Asp
180 185 190

Asp Asn Leu Val Tyr His Ile Leu Arg Lys His Ser Ala Lys Arg Cys
195 200 205

Gly Cys Ile
210